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A COMPARISON OF MICROCOSM AND BIOASSAY TECHNIQUES FOR  
ESTIMATING ECOLOGIC. (U) OLD DOMINION UNIV NORFOLK VA  
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APPLIED MARINE RESEARCH LABORATORY  
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NORFOLK, VIRGINIA

A COMPARISON OF MICROCOSM AND BIOASSAY  
TECHNIQUES FOR ESTIMATING ECOLOGICAL  
EFFECTS FROM OPEN OCEAN DISPOSAL OF  
CONTAMINATED DREDGED SEDIMENTS

By

Raymond W. Alden III  
Arthur J. Butt  
Susanne S. Jackman  
Guy J. Hall  
Robert J. Young, Jr.

Supplemental Contract Report  
For the period ending September 1984

Prepared for the  
Department of the Army  
Norfolk District, Corps of Engineers  
Fort Norfolk, 803 Front Street  
Norfolk, Virginia 23510

Under  
Contract DACW65-81-C-0051  
Work Order No. 16

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Report B- 50

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REPORT

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1a. REPORT SECURITY CLASSIFICATION Unclassified		5. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release, distribution unlimited.	
2a. SECURITY CLASSIFICATION AUTHORITY		5. MONITORING ORGANIZATION REPORT NUMBER(S) B-50	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE		7a. NAME OF MONITORING ORGANIZATION U.S. Army Corps of Engineers, Norfolk District	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)		7b. ADDRESS (City, State, and ZIP Code) Norfolk, Virginia 23510-1096	
6a. NAME OF PERFORMING ORGANIZATION Old Dominion University Applied Marine Research Laboratory	6b. OFFICE SYMBOL (If applicable) NAOPL; NAOEN	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DACW65-81-C-0051	
6c. ADDRESS (City, State, and ZIP Code) Norfolk, VA 23508		10. SOURCE OF FUNDING NUMBERS	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Corps of Engineers, Norfolk District		PROGRAM ELEMENT NO.	PROJECT NO.
8b. OFFICE SYMBOL (If applicable) NAOPL; NAOEN		TASK NO.	WORK UNIT ACCESSION NO.
8c. ADDRESS (City, State, and ZIP Code) Norfolk, Virginia 23510-1096		11. TITLE (Include Security Classification) A Comparison of Microcosm and Bioassay Techniques for Estimating Ecological Effects from Open Ocean Disposal of Contaminated Dredged Sediments	
12. PERSONAL AUTHOR(S) Alden, R.W., III, A.J. Butt, S.S. Jackman, G.J. Hall, and R.J. Young, Jr.			
13a. TYPE OF REPORT Final	13b. TIME COVERED FROM TO	14. DATE OF REPORT (Year, Month, Day) 1985, March	15. PAGE COUNT 45
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	ecological impact, bioassay, microcosm, Norfolk Harbor and Channels, dredging, Southern Branch Elizabeth River, toxicity, comparison study, sediment quality	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) Results of study reflect the fact that the more natural conditions in the microcosms stimulate activity in the test organisms (bivalves in this case) that would otherwise enter a resting phase when exposed to contaminated sediments in the static bioassays. Microcosms may more accurately portray what is happening under natural field conditions. This looks like a good tool for future assessments.			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Craig L. Seltzer		22b. TELEPHONE (Include Area Code) (804) 441-3767/827-3767	22c. OFFICE SYMBOL NAOPL-R

APPLIED MARINE RESEARCH LABORATORY  
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**A COMPARISON OF MICROCOSM AND BIOASSAY  
TECHNIQUES FOR ESTIMATING ECOLOGICAL EFFECTS  
FROM OPEN OCEAN DISPOSAL  
OF CONTAMINATED DREDGED SEDIMENTS**

By

Raymond W. Alden III\*, Arthur J. Butt\*\*,  
Susanne S. Jackman\*\*\*, Guy J. Hall\*\*\*\*,  
and Robert J. Young, Jr.\*\*\*\*\*

**INTRODUCTION**

The potential ecological impact of open ocean disposal of dredged material must be assessed on a site by site basis. A variety of research methods can be employed for this assessment. Static bioassays have been and continue to be the most common means for biologically evaluating the toxicity of dredged sediments. The validity of bioassay techniques in effectively assessing the potential ecological impact of ocean disposal of dredged materials is open to question. This report deals specifically with results of a study designed to assess the relative effectiveness of standard bioassays and multiple species microcosms in the evaluation of the suitability of dredged materials for open ocean disposal. *Keywords: Norfolk Harbor and*

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*Channels; sediment quality, ←*

Typically, static bioassays expose test organisms to the sediments in question for a specified length of time. Based on recorded mortalities, conclusions are made as to the potential lethality of the dredged material. A series of extended liquid, suspended solid and solid phase bioassays are designed to evaluate not only the toxicity of the sediments fractions, but the bioaccumulation potential of the toxins in the test organisms as well (EPA/COE Implementation Manual, 1978). However, such experiments are often limited to very simple community structures and only a few abiotic parameters are usually monitored. An experimental design is needed to more closely "mimic" the in situ field conditions of the impacted area.

Microcosm experiments are expected to be more realistic indicators of sediment toxicity. They more closely simulate natural environmental conditions by testing indigenous populations from the study area(s). Entire assemblages of phytoplankton, zooplankton and benthos can be monitored following exposure to the dredged materials. Also, a greater variety of physical and water quality parameters can be evaluated for changes between pre- and post-dump conditions. These measurements, in turn, can be compared to the actual field baseline data (Alden, 1984). Moreover, bioaccumulation potential of toxins in biota exposed to simulated field conditions can be determined from the microcosm experimental design.

The present study details a direct comparison of the relative effectiveness of static bioassays and multiple species microcosms. The experimental design involved a "blind" test of sediments previously shown to be toxic mixed in a "dilution

series" with pristine sediments. The two toxicity testing techniques were evaluated in terms of their effectiveness in correctly identifying the relative toxicity of the sediment series.

## METHODS AND MATERIALS

### Study Area and Sediment Preparation

The Elizabeth River is the principal deepwater navigational channel in the Port of Hampton Roads, Virginia. The Port is the one of the world's largest natural harbor areas and the surrounding estuarine systems are highly industrialized. Hampton Roads is located in the metropolitan area that includes the cities of Norfolk, Virginia Beach, Portsmouth, Hampton and Newport News (Fig. 1a) and is the site of the largest military port in the world.

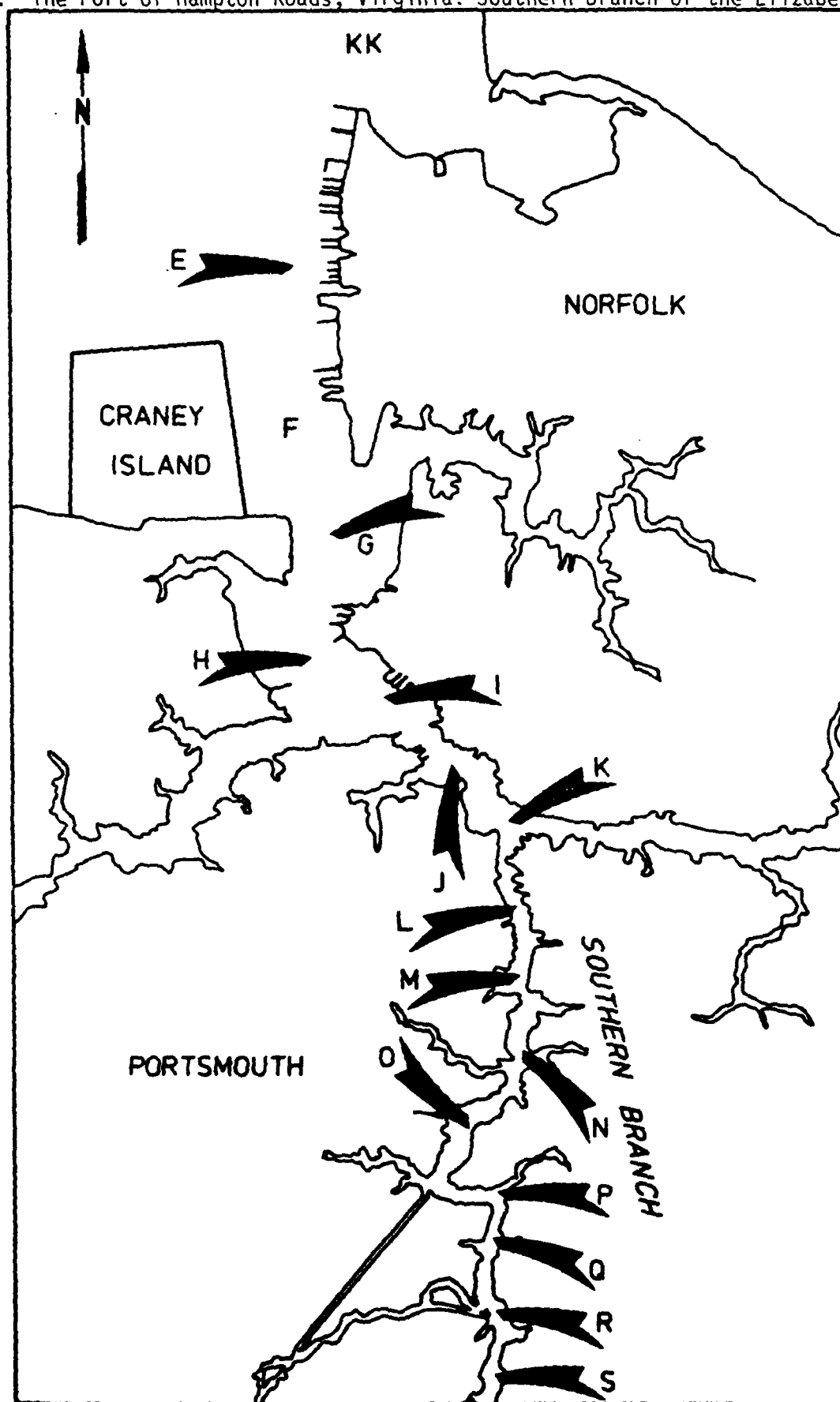
The River receives many point and nonpoint sources of pollution including input from sewage treatment facilities, shipyards, fertilizer plants, oil industries, cement manufacturers, creosote plants, chemical manufacturers and utilities. The water quality is generally defined as poor. Sediments from various parts of the River have been defined as being grossly polluted (COE, 1974) and fishing and swimming activities have been banned for large portions of the Elizabeth River for decades.

Previous studies have shown that the sediments from Stations M and O of the Southern Branch of the Elizabeth River (Fig. 1b) to be heavily contaminated with heavy metals and polynuclear aromatic hydrocarbons (PNAH's) (Alden et al., 1981; Alden and Young, 1982; Alden et al., 1984; Alden and Young, 1984; Alden and Hall, 1984; Alden et al., 1985). Test sediments from these two sites were collected in 18 l polyethylene buckets inserted into a stainless steel bucket dredge. Immediately after collection, the

The map illustrates the coastal region of Virginia, focusing on the Chesapeake Bay and its tributaries. Key features include:

- Water Bodies:** James River, Chesapeake Bay, Thimble Shoal Channel, Cape Henry Channel, Elizabeth River, and Hampton Roads Harbor.
- Landmarks and Cities:** Newport News, Hampton, Norfolk, Portsmouth, and Virginia Beach.
- Infrastructure:** Chesapeake Bay Bridge Tunnel, Craney Island, and various navigational channels (AA, BB, CC, DD, EE, FF, GG, HH, II, KK, LL, MM, NN, OO, PP, QQ, RR, SS, TT, UU, VV, WW, XX, YY, ZZ).
- Orientation:** A compass rose indicates North (N).
- Inset Map:** A small map of the United States shows the location of the study area on the East Coast, near the Atlantic Ocean.

Figure 1b. The Port of Hampton Roads, Virginia: Southern Branch of the Elizabeth River.



polyethylene insert was removed and sealed by a snap top. A composite of the two sites was made by mixing the sediments in a 1:1 ratio and rotating them in a stainless steel drum. A sediment composite from a nonindustrialized (or control) source was mixed with the Elizabeth River sediments (ERS). The nonindustrialized sediments, similar in particle size and organic content to the Elizabeth River materials, were obtained from the Eastern Shore near Cape Charles, Virginia. The "pristine" sediment composite was homogenized as above and mixed with the "toxic" sediments to form a series: 0%, 25%, 50% and 100% concentrations. The concentrations were coded by a person not involved in the project and the identities of the sediment concentrations were not known by the investigation team until after the statistical analysis/interpretation of the results. The sediments were frozen to kill the indigenous benthic communities.

### **Bioassay Methods**

Liquid, suspended solid, and solid phase bioassays were conducted in 30 l aquaria using artificial seawater at 30<sup>o</sup>/oo, 20<sup>o</sup>C and with a 14:10 day/night cycle. These bioassays followed standard procedures outlined in the EPA/COE Implementation Manual (1978). The test organisms were the copepod Acartia tonsa, grass shrimp Palaemonetes pugio, the sheepherd minnow Cyprinodon variegatus, the sand worm Nereis virens, and the hard clam Mercenaria mercenaria. The copepods and grass shrimp were collected from a nonindustrialized habitat while the fish, worms, and clams were purchased from a commercial supply house. All test organisms except the copepods were placed in a holding tank

(300/00, artificial seawater) and held for no more than two weeks. Sediments from the proposed Norfolk Disposal Site (NDS) were used as reference sediments for the acclimatization in the solid phase experiments. The shrimp, fish and copepods were used in the liquid and suspended solid phase tests, while the shrimp, worms and clams were employed in the solid phase experiments. Mortalities of test organisms were recorded at the end of the tests. The clams were purged in clean seawater for 24 hours and frozen until analysis for bioaccumulation potential.

Those clams analyzed for trace metals were dried at 60°C and weighed. They were wet ashed using HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. Sediments samples analyzed for metals were air dried, weighed, and digested using HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. The digestates of both tissue and sediment were brought to volume with deionized water and stored in polyethylene bottles. The tissues were analyzed for copper (Cu), cadmium (Cd), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn).

The polynuclear aromatic hydrocarbons (PNAH's) in tissues and sediments were analyzed according to methods recommended by EPA (1980b) and Brown et al. (1980), respectively. The cleaned extracts were analyzed on a capillary gas chromatography system fitted with a flame ionization detector (FID) and a data microprocessor. The PNAH's were quantitated against an internal standard (1,1-binaphthyl) which was added to each of the samples at the beginning of the extraction process. Representative samples were analyzed by GC/MS to confirm the identity of toxins.



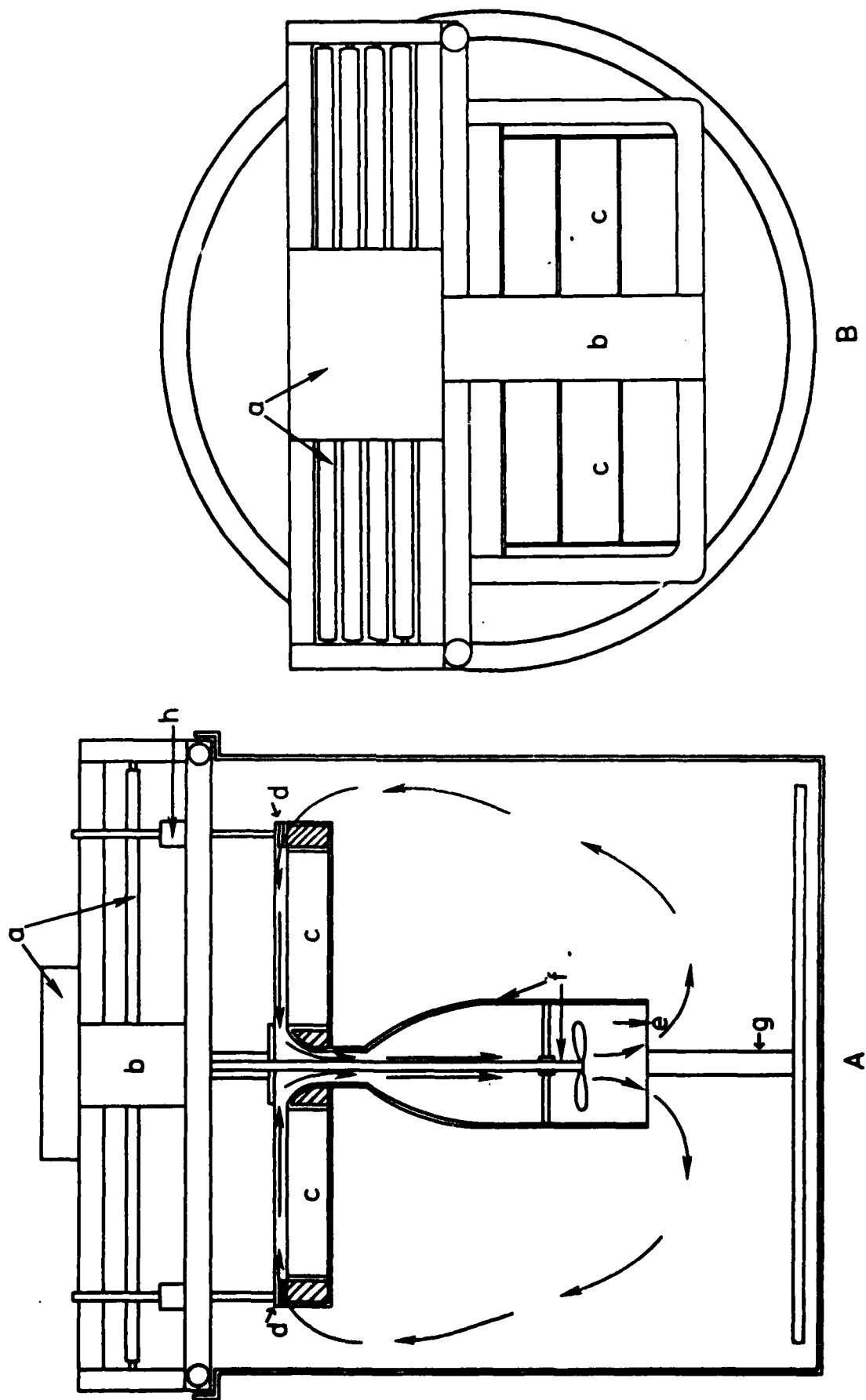
## Microcosms

Microcosms were preformed in 1500 liter polyethylene barrels filled with natural seawater maintained at 20°C and a 14:10 day/night cycle. The barrels contained two benthic trays, each with three chambers, an additional tray for a population of clams, and a light source (Figs. 2a,b). Two types of water circulating devices were operational in each barrel. One circulated the entire barrel water to simulate oceanic currents and maintain the plankton in suspension. The second device drew water over the benthic trays to simulate epibenthic circulation.

The seawater was collected at the mouth of the Chesapeake Bay at approximately 30‰ salinity. Zooplankton tows were also taken at the Bay site and used for microcosm barrel enrichment. Sediment samples, with their indigenous benthos, were collected with a Shipek grab in a sandy bottom area near Cape Charles, VA. All field samples were transported to the laboratory as soon as possible for dispensing into the microcosm barrels. Seawater and zooplankton samples were distributed to the barrels by a gravity-flow ducting system to minimize organismal damage. Sediments with benthic communities were placed in the sediment trays and allowed to equilibrate for 96 hours. Defaunated sediments were placed in the additional trays along with a population of the clams for the bioaccumulation experiments. After equilibration, defaunated test sediments were dumped on top of benthic and clam trays. After the dump, the benthic trays were covered and not further disturbed.

Following the 10 day experimental period, the benthic organisms were harvested by sieving, preserved in formalin-rose

Figure 2. Microcosm chamber (a. x-sectional view; b. plane view) with lightbank (a), circulation motor (b), sediment holding trays (c), water inflow channel (d), tray circulation outflow (e), tray circulation rotor (f), barrel circulation rotor (g), and tray support screws for adjusting tray depth in barrel.



bengal, sorted and identified. The zooplankton communities were sampled with a 3" diameter Wisconsin style plankton net (150 micron mesh). The harvested clams were placed in clean seawater and treated in the same manner as bioassay clams for the evaluation of body burdens of toxins.

## RESULTS

### Biological Effects

The biological data (i.e. mortalities and relative species survival from bioassay and microcosms, respectively) indicated that none of the sediments were toxic. The clam and minnow populations displayed 100% survival in all experiments. The shrimp and worms also displayed low mortalities for all bioassay conditions ( $\bar{x} \leq 10\%$ ). Likewise, the community structures of the benthos and the zooplankton were shown by MANOVA not to be significantly different ( $\alpha=0.05$ ) between any of the experimental conditions in the microcosms.\*

The only species to exhibit elevated mortalities in the bioassay was the copepod Acartia tonsa (Table 1). Mortalities for all concentrations of the suspended solid elutriates of all sediments were always very high, if not total. The copepods exposed to the liquid phase fractions of all sediments displayed mortalities which increased with greater concentrations. The overall mortalities in the liquid phase series for the two sediments representing the two highest concentrations of the ERS (i.e. B-50%, C-100%) appeared somewhat higher than in the other two experiments. The control mortalities were also somewhat elevated in the copepod tests, but never to the levels observed in the corresponding experimental tanks.

\*For space considerations, the species lists and abundance data for these communities in each treatment are not shown. These data are available from the authors upon request.

Table 1. Mean percent mortalities (standard errors) of Acartia tonsa in liquid and suspended solid phase bioassays.

<u>Treatment*</u>	<u>Concentration of Elutriate</u>			
	<u>Control</u>	<u>10%</u>	<u>50%</u>	<u>100%</u>
A Liquid	10 (5.8)	30 (0)	33 (3.3)	83 (8.8)
A Suspended solids	10 (5.8)	100 (0)	100 (0)	100 (0)
B Liquid	53 (16.7)	83 (16.7)	93 (6.7)	100 (0)
B Suspended solids	60 (20.8)	96.7 (3.3)	100 (0)	100 (0)
C Liquid	33 (6.7)	66 (6.7)	83 (3.3)	100 (0)
C Suspended solids	33 (5.8)	100 (0)	100 (0)	100 (0)
D Liquid	15 (5.0)	77 (6.7)	83 (3.3)	87 (8.8)
D Suspended solids	23 (6.7)	80 (10)	100 (0)	100 (0)

\* The percent of Elizabeth River sediments in the "blind" series were as follows:  
A - 0%; B - 50%; C - 100%; and D - 25%.

## Sediments

Duplicate sediment samples were analyzed for Cu, Cr, Cd, Fe, Mn, Ni, Pb and Zn. The sediment concentrations of Cu, Zn, Pb and Mn were lowest in the reference sediment (NDS) and increased significantly (ANOVA and Duncan's tests;  $\alpha=0.05$ ) with increasing amounts of Elizabeth River sediment (ERS) (Table 2). The reference sediment concentrations of Cr were significantly lower than the ERS fraction. There was no significant differences between 0% and 25% ERS and 25% and 50% fractions, respectively; however, the 100% ERS sediments were the highest. The iron content was lowest in the reference sediment and increased significantly in the 0% and 25% ERS, the 50%, and 100% ERS. Nickel was lowest in the reference sediment and was significantly different from all other sediment types. The 0%, 25%, 50% and 100% ERS were difficult to distinguish based on Ni content, indicating that the Ni levels were similar in the Elizabeth River and Eastern Shore sediments. There was no significant difference in the Cd concentration between the reference, 0% and 25% ERS. The 50% and 100% ERS were significantly different from the other sediment types but not from each other. The Cd levels appear to be only slightly elevated in the ERS compared to the levels in the reference and Eastern Shore sediments.

Sediment samples from the experimental dilution series were also analyzed for PNAH's (Table 3). The levels of PNAH's were clearly related to the concentration of ERS in the sediments. Moderately high levels of PNAH's (ppm) were observed in these experimental sediments. Lower levels were observed in the Eastern

Table 2. Mean concentrations (standard errors) of metals ( $\mu\text{g/g}$ ) in sediments employed in the bioassays and microcosms. Statistically homogeneous ( $\alpha=0.05$ ) subset 5 based on Duncan's test comparisons are indicated by letters.

Metal	Treatment (% ERS)				
	A (0%)	B (50%)	C (100%)	D (25%)	Ref (NDS)
Cu	41.2 <sup>b</sup> (2.3)	92.9 <sup>d</sup> (5.8)	172.9 <sup>e</sup> (1.2)	76.3 <sup>c</sup> (1.7)	0.0 <sup>a</sup> (-)
Cd	0.100 <sup>a</sup> (0.001)	1.390 <sup>bc</sup> (0.444)	2.250 <sup>c</sup> (0.406)	0.938 <sup>ab</sup> (0.011)	0.0 <sup>a</sup> (-)
Cr	42.6 <sup>b</sup> (0.2)	49.7 <sup>c</sup> (0.1)	63.8 <sup>d</sup> (2.7)	45.8 <sup>bc</sup> (1.3)	0.0 <sup>a</sup> (-)
Fe	29,469 <sup>b</sup> (118)	33,180 <sup>c</sup> (44)	35,396 <sup>d</sup> (238)	29,023 <sup>b</sup> (333)	1,059 <sup>a</sup> (91)
Mn	234.3 <sup>b</sup> (0.9)	270.8 <sup>d</sup> (5.8)	330.2 <sup>e</sup> (2.2)	253.2 <sup>c</sup> (2.9)	10.2 <sup>a</sup> (1.0)
Ni	33.7 <sup>bc</sup> (0.1)	34.7 <sup>bc</sup> (1.2)	37.0 <sup>c</sup> (1.4)	32.1 <sup>b</sup> (1.6)	0.0 <sup>a</sup> (-)
Pb	39.6 <sup>b</sup> (0.2)	89.3 <sup>d</sup> (5.7)	155.6 <sup>e</sup> (4.5)	72.3 <sup>c</sup> (0.8)	0.0 <sup>a</sup> (-)
Zn	151.8 <sup>b</sup> (0.6)	274.5 <sup>d</sup> (7.6)	474.9 <sup>e</sup> (7.4)	216.7 <sup>c</sup> (1.0)	3.0 <sup>a</sup> (3.0)

Note: Those values which were below detection limits were represented by zero for statistical analyses.

Table 3. Concentrations of PNAH's (ng/g) in sediments employed in the bioassays and microcosms.

PNAH's	Treatment (% ERS)				Ref (NDS)
	A (0%)	B (50%)	C (100%)	D (25%)	
Naphthalene (N)	BDL	141.5	241.3	BDL	BDL
Acenaphthylene (Acy)	BDL	BDL	BDL	BDL	BDL
Acenaphthene (Ace)	10.6	1,431.9	1,932.4	693.2	BDL
Fluorene (F)	11.3	1,878.6	2,461.8	1,085.9	BDL
Phenanthrene (Ph)	28.5	6,941.4	9,255.8	4,880.4	10.8
Anthracene (A)	44.3	4,429.7	4,764.2	1,980.5	10.8
Fluoranthene (F1)	244.8	6,829.0	7,983.6	5,223.8	9.4
Pyrene (Pyre)	134.3	4,410.0	4,921.9	3,322.1	BDL
Benzo(a)anthracene (B(a)A)	429.3	3,691.9	4,707.1	2,614.2	22.8
Chrysene (Ch)	265.1	3,825.6	5,224.6	2,744.9	BDL
Dibenzanthracene (Di(b)A)	BDL	893.7	644	728.5	BDL
Benzo(ghi)perylene (B(ghi)P)	BDL	BDL	BDL	BDL	BDL
Benzo(a)pyrene (B(a)P)	BDL	3,543.9	4,852	3,460.0	BDL
Benzofluoranthene(s) (BF1)	BDL	12,841.5	18,628	10,047.7	BDL
Indeno(1,2,3-cd)pyrene (IP)	BDL	862.7	945	561.6	BDL



Shore sediments. Those PNAH's observed above detection levels in these "clean" sediments were 1-2 orders of magnitude below the ERS. Only trace levels of a very few of the PNAH's were observed in the reference (NDS) sediments.

### Body Burdens of Toxins

Tissue metal analyses were performed on two replicates from each replicate microcosm barrel. This yielded a total of four replicates per sediment type. Five replicate clams were analyzed per sediment type used in the bioassay, one from each aquarium. The organismal metal data labeled as reference are background clams collected and frozen immediately upon arrival in the laboratory. Four reference clams were analyzed from both the microcosm batch and the bioassay batch.

Copper and zinc concentrations in experimental clams exhibited similar trends with respect to sediment type exposure. There was no significant Cu or Zn bioaccumulation in bioassay-exposed clams compared to controls (Table 4). There was differential Cu bioaccumulation observed in microcosm-exposed organisms. The following were statistically similar groups for Cu: reference 0%, 25%, and 50%; 50% and 100%. The microcosm-exposed clams were grouped for Zn as follows: reference 0%, 25% and 50%; 50%, and 100%.

There was no significant bioaccumulation of Fe or Mn from test sediments in the bioassay-exposed organisms (Table 4). Clams from the microcosm had significantly elevated Fe levels for

Table 4. Mean concentrations (standard errors) of metals ( $\mu\text{g/g}$ ) in *Mercenaria mercenaria* tissues from the microcosms and bioassays. Statistically homogeneous ( $\alpha=0.05$ ) subsets based on Duncan's test comparisons are indicated by letters.

Metal	Microcosm Treatment (% ERS)					Bioassay Treatment (% ERS)				
	A (0%)	B (50%)	C (100%)	D (25%)	Ref (NDS)	A (0%)	B (50%)	C (100%)	D (25%)	Ref (NDS)
Cu	34.87 <sup>b</sup> (0.20)	38.24 <sup>bc</sup> (6.44)	57.24 <sup>c</sup> (4.49)	31.67 <sup>ab</sup> (13.34)	12.00 <sup>a</sup> (1.42)	9.16 <sup>a</sup> (2.63)	11.14 <sup>a</sup> (1.04)	11.16 <sup>a</sup> (0.66)	11.02 <sup>a</sup> (0.51)	6.94 <sup>a</sup> (3.14)
Cd	1.11 <sup>a</sup> (0.32)	0.61 <sup>a</sup> (0.20)	0.82 <sup>a</sup> (0.06)	0.86 <sup>a</sup> (0.36)	1.44 <sup>a</sup> (0.36)	0.15 <sup>a</sup> (0.15)	0.57 <sup>a</sup> (0.15)	0.64 <sup>a</sup> (0.16)	0.77 <sup>a</sup> (0.28)	0.52 <sup>a</sup> (0.22)
Fe	468.99 <sup>b</sup> (18.32)	666.87 <sup>bc</sup> (87.44)	813.21 <sup>c</sup> (111.85)	655.88 <sup>bc</sup> (71.16)	164.63 <sup>a</sup> (10.49)	194.06 <sup>a</sup> (49.51)	241.74 <sup>a</sup> (15.71)	228.81 <sup>a</sup> (5.46)	225.36 <sup>a</sup> (18.80)	210.04 <sup>a</sup> (87.93)
Mn	18.19 <sup>b</sup> (3.23)	20.06 <sup>b</sup> (4.57)	23.05 <sup>b</sup> (3.21)	17.95 <sup>b</sup> (0.84)	7.53 <sup>a</sup> (2.97)	20.25 <sup>a</sup> (6.88)	14.92 <sup>a</sup> (3.34)	11.78 <sup>a</sup> (1.61)	11.71 <sup>a</sup> (0.88)	11.61 <sup>a</sup> (5.40)
Ni	11.25 <sup>ab</sup> (1.66)	11.19 <sup>ab</sup> (2.41)	7.21 <sup>a</sup> (1.76)	15.96 <sup>c</sup> (3.92)	7.78 <sup>a</sup> (1.12)	8.90 <sup>ab</sup> (3.23)	14.97 <sup>bc</sup> (1.86)	20.07 <sup>c</sup> (1.77)	18.19 <sup>c</sup> (1.03)	5.95 <sup>a</sup> (2.89)
Zn	189.38 <sup>bc</sup> (15.79)	191.54 <sup>bc</sup> (4.98)	233.81 <sup>c</sup> (39.18)	163.38 <sup>ab</sup> (5.72)	125.53 <sup>a</sup> (13.40)	95.42 <sup>a</sup> (25.81)	127.50 <sup>a</sup> (6.56)	126.17 <sup>a</sup> (3.47)	110.15 <sup>a</sup> (4.60)	70.18 <sup>a</sup> (29.18)
Pb	9.13 <sup>a</sup> (9.13)	22.96 <sup>a</sup> (14.21)	36.54 <sup>a</sup> (23.78)	15.72 <sup>a</sup> (11.35)	BDL <sup>a</sup>	BDL <sup>a</sup>	7.25 <sup>a</sup> (4.45)	BDL <sup>a</sup>	3.79 <sup>a</sup> (3.79)	BDL <sup>a</sup>

all test sediment concentrations. Likewise, Mn content from the microcosm clams were greater than the reference organisms, but there was little difference between the experimental concentrations. There were no apparent trends for Ni concentration in microcosm-exposed animals. The bioassay-exposed clams had the highest Ni levels in those organisms exposed to the 25%, 50% and 100% ERS. There was no Cd bioaccumulation pattern observed in either bioassay or microcosm-exposed clams.

Several different models emerged for the various metals when the tissue data were analyzed by multiple regression analysis. A similar pattern appeared for Cu, Zn and Fe levels in the clams compared by experiment and sediment type (Figs. 3 a, b, and c). Microcosm-exposed clams had higher concentrations than bioassay exposed clams for all test sediments. There were no significant difference in these three metal levels of the reference animals from the two batches. The Cu and Zn tissue concentrations increased significantly with increasing ERS concentration. The Fe levels were significantly higher in the microcosm-exposed clams than the bioassay-exposed clams. There was a significant correlation between Fe content and sediment concentration for the clams from the microcosm. The clams from the microcosm exhibited a positive relationship between Mn body burden and sediment concentration, while that observed for the bioassay organisms was slightly negative (Fig. 3d). The results of the multiple regression analysis showed no significant relationships.

The PNAH's in clams exposed to the sediment series in the bioassays were seldom above detection levels (Table 5). Even for those cases when certain PNAH's were detected the experimental

Fig. 3. Multiple regression models for the metals in tissues of the hard clam M. mercenaria microcosms and bioassays. The sediment types used were: Ref. 0%, 25%, 50% and 100% ERS. The metals were: a) Cu; b) Zn; c) Fe; d) Mn; and e) Ni.

Figure 3a.

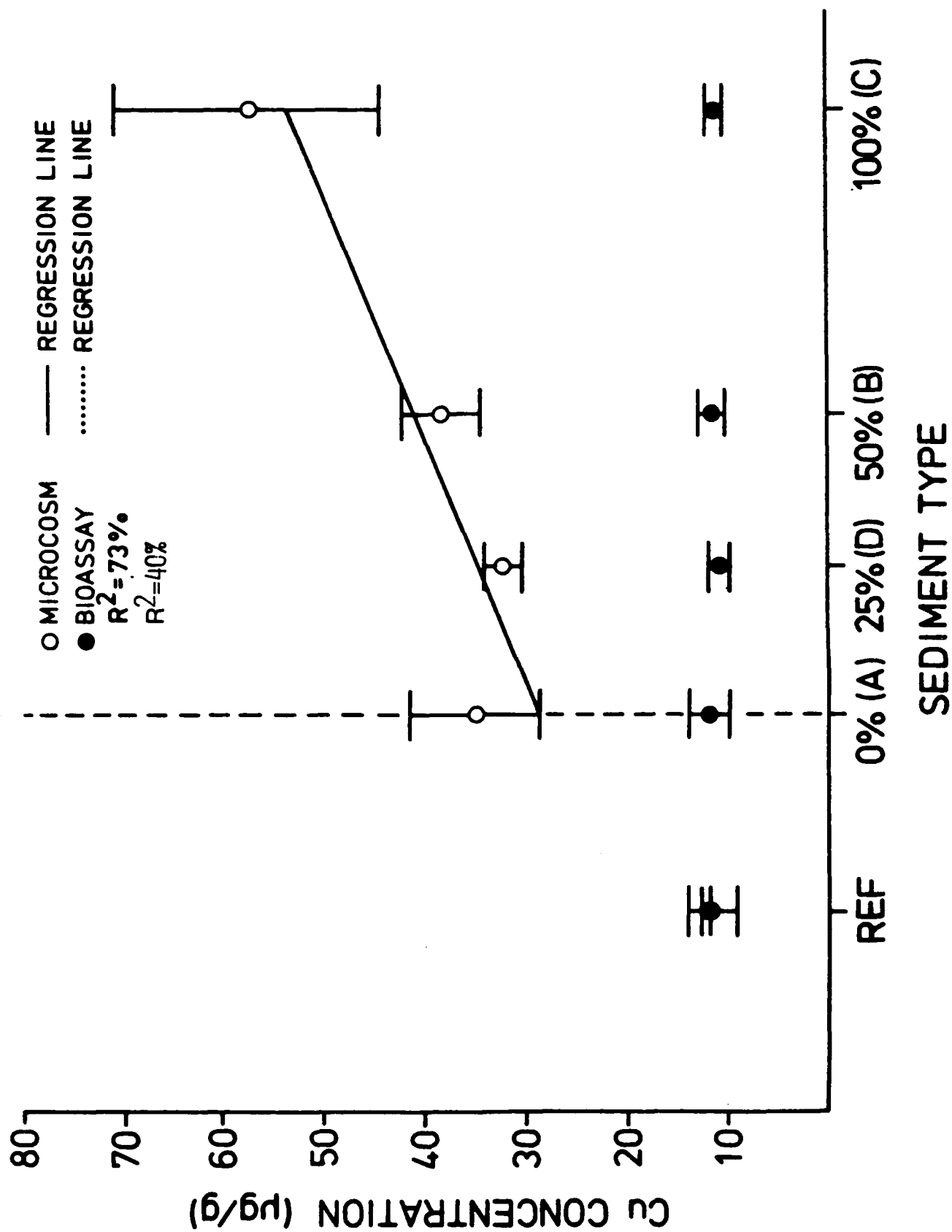


Figure 3b.

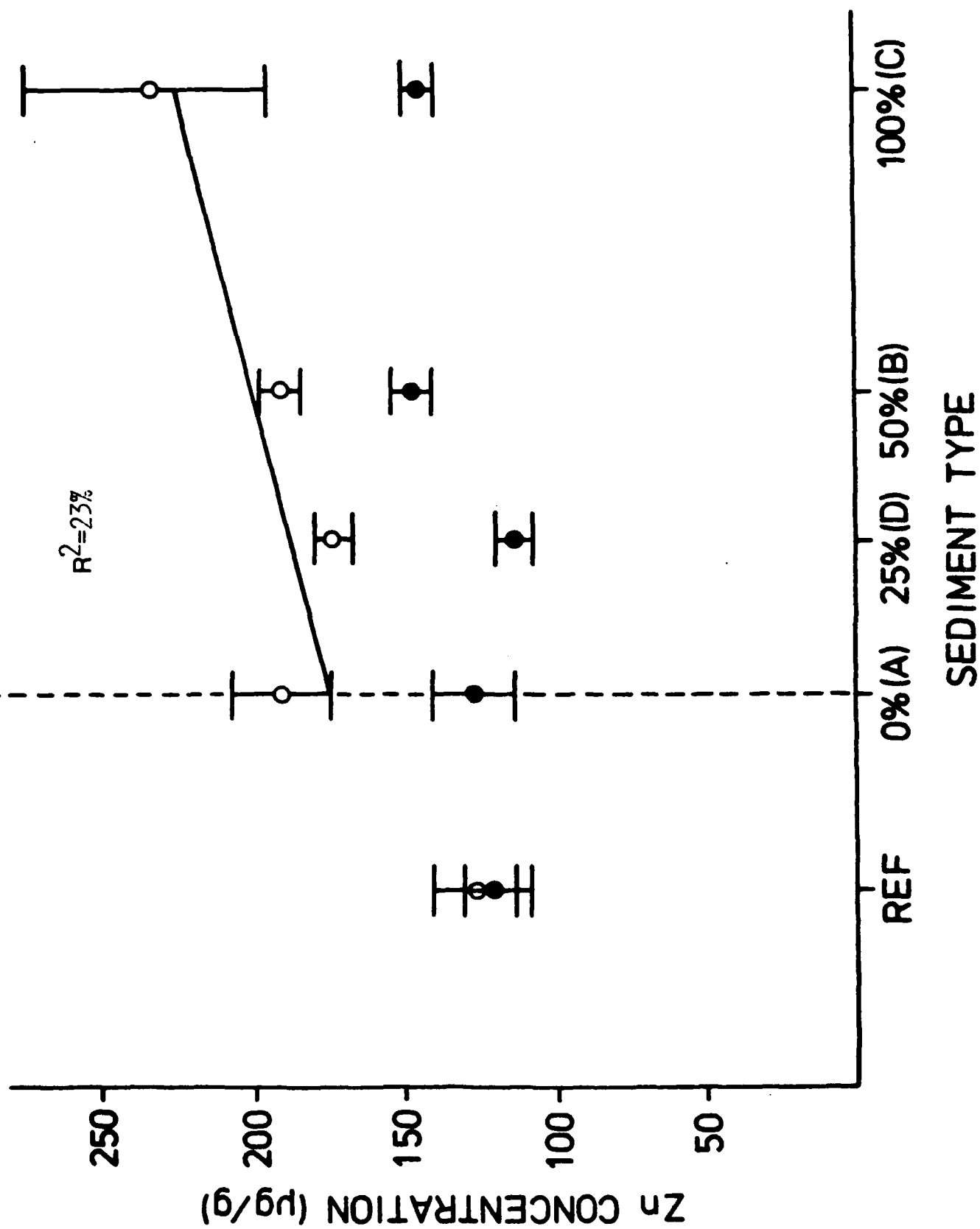


Figure 3c.

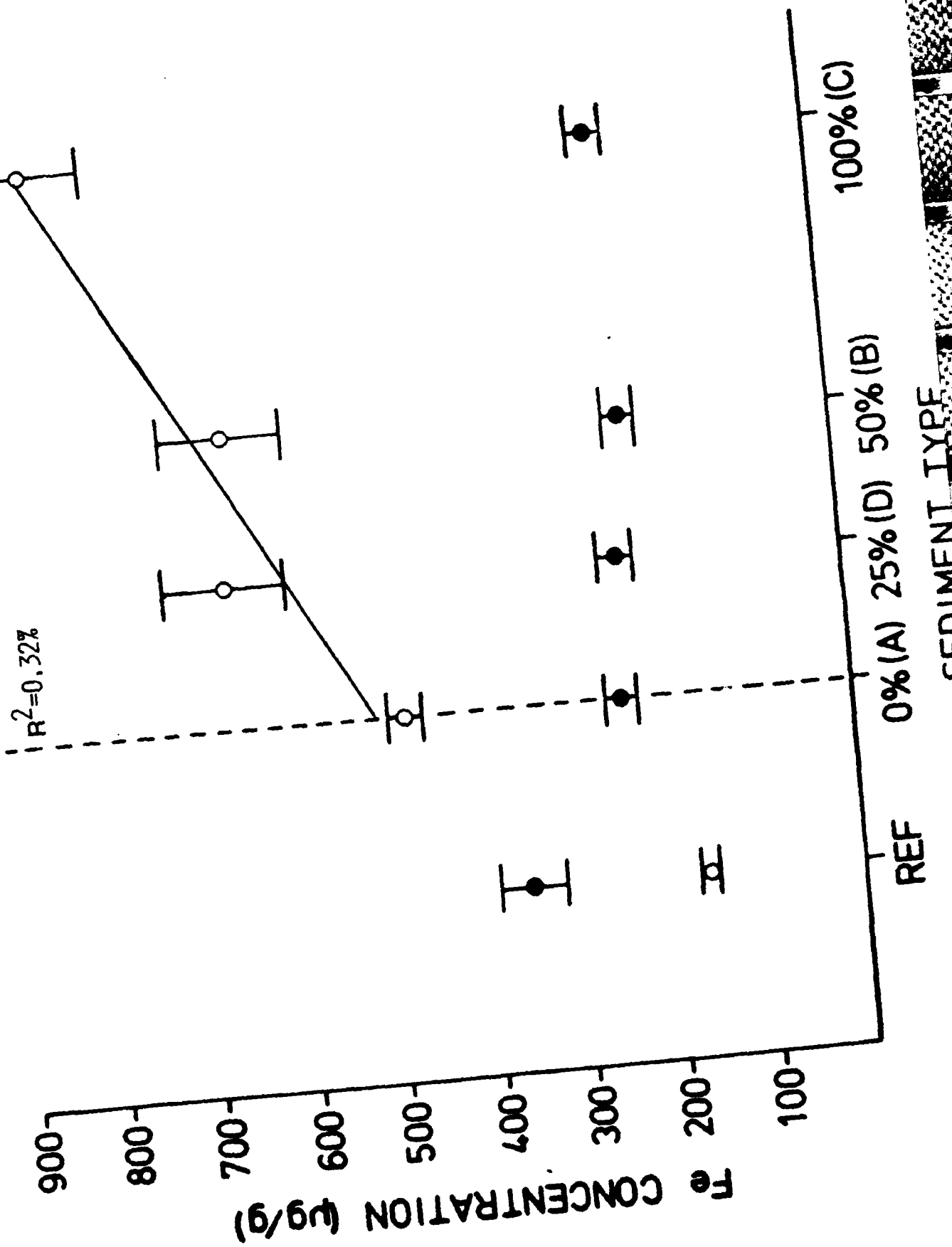


Figure 3d.

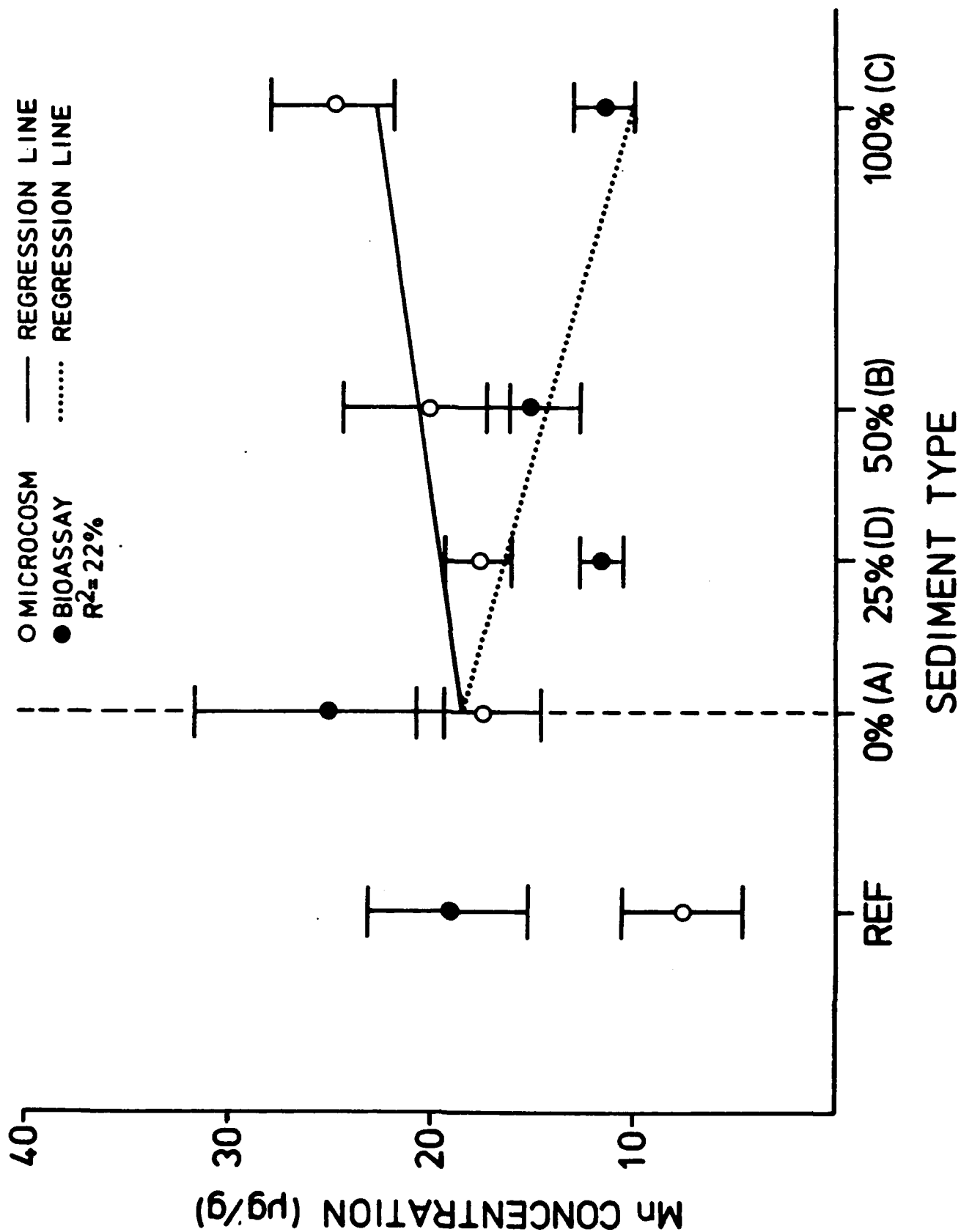




Table 5. Mean concentrations (standard errors) of PNAH's (ng/g) in *Mercenaria mercenaria* tissues from microcosm/bioassay tests. Statistically homogeneous ( $\alpha=0.05$ ) subsets based on Duncan's test comparisons are indicated by letters.

PNAH's	Microcosm Treatment (% ERS)					Bioassay Treatment (% ERS)				
	A (0%)	B (50%)	C (100%)	D (25%)	Ref (NDS)	A (0%)	B (50%)	C (100%)	D (25%)	Ref (NDS)
Naphthalene (N)	354.25 <sup>a</sup> (87.14)	299 <sup>a</sup> (70.71)	242.47 <sup>a</sup> (73.24)	351.72 <sup>a</sup> (96.86)	210.55 <sup>a</sup> (135.71)	<37	<37	<37	<37	<37
Acenaphthylene (Acy)	<15	<15	<15	<15	<15	<15	<15	<15	<15	<15
Acenaphthene (Ace)	<12	<12	<12	<12	<12	20.14 <sup>a</sup> (20.14)	<12	<12	30.62 <sup>a</sup> (30.62)	<12
Fluorene (F)	< 5	< 5	< 5	< 5	< 5	38.85 <sup>a</sup> (38.85)	< 5	< 5	< 5	< 5
Phenanthrene (Ph)	< 5	35.69 <sup>a</sup> (15.52)	99.81 <sup>b</sup> (27.00)	25.68 <sup>a</sup> (19.01)	< 5	< 5	< 5	< 5	< 5	< 5
Anthracene (A)	< 5	9.94 <sup>ab</sup> (5.24)	17.32 <sup>b</sup> (4.59)	7.25 <sup>ab</sup> (4.28)	< 5	84.12 <sup>a</sup> (84.12)	112.21 <sup>a</sup> (112.21)	125.48 <sup>a</sup> (125.58)	103.13 <sup>a</sup> (103.13)	108.65 <sup>a</sup> (62.73)
Fluoranthene (F1)	< 5	278.34 <sup>ab</sup> (143.39)	536.59 <sup>b</sup> (147.82)	51.78 <sup>a</sup> (41.63)	< 5	64.39 <sup>a</sup> (64.39)	< 5	< 5	< 5	< 5
Pyrene (Pyre)	< 5	< 5	100.18 <sup>a</sup>	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Benzo(a)anthracene (B(a)A)	<17	39.37 <sup>a</sup> (25.82)	74.65 <sup>a</sup> (35.49)	56.39 <sup>a</sup> (30.23)	18.93 <sup>a</sup> (12.65)	<17	<17	<17	<17	<17
Chrysene (Ch)	28.67 <sup>a</sup> (28.67)	93.73 <sup>a</sup> (49.31)	138.48 <sup>a</sup> (46.77)	20.48 <sup>a</sup> (8.93)	91.86 <sup>a</sup> (52.52)	<25	<25	<25	<25	<25
Dibenzanthracene (D1(b)A)	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50

Table 5. (Continued)

PNAH's	Microcosm Treatment (% ERS)					Bioassay Treatment (% ERS)				
	A (0%)	B (50%)	C (100%)	D (25%)	Ref (NDS)	A (0%)	B (50%)	C (100%)	D (25%)	Ref (NDS)
Benzo(ghi)perylene (B(ghi)P)	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50
Benzo(a)pyrene (B(a)P)	<12	<12	<12	<12	<12	<12	<12	<12	<12	<12
Benzo(b)Fluoranthene (B(b)F1)	<28	<28	<28	<28	<28	<28	<28	<28	<28	<28
Benzo(k)Fluoranthene (B(k)F1)	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
Indeno(1,2,3-cd)pyrene (IP)	<51	<51	<51	<51	<51	<51	<51	<51	<51	<51

concentrations were never significantly (ANOVA, Duncan's test,  $\alpha=0.05$ ) greater than reference levels. On the other hand, the clams exposed to the same sediment series in the microcosms exhibited significantly elevated levels of phenanthrene (100% ERS), anthracene (25%, 50%, 100% ERS), and fluoranthene (50% and 100% ERS). The levels of pyrene, benzanthrane and chrysene also appeared to be somewhat elevated in the 100% ERS microcosm clams, but the trend was not statistically significant.

The multiple regression models for phenanthrene, fluoranthene, benzanthrane, and chrysene all indicated significant relationships between the body burdens of the clams and the concentration of the ERS (Fig. 4). The body burden of clams from the bioassays did not display any significant relationships with the sediment concentrations. Therefore, there appeared to be a significant bioaccumulation potential associated with the microcosm condition not found in the bioassays.

Pb CONCENTRATION (ng/g)

$R^2 = 56\%$

— regression line

○ microcosm

● bioassay

REF

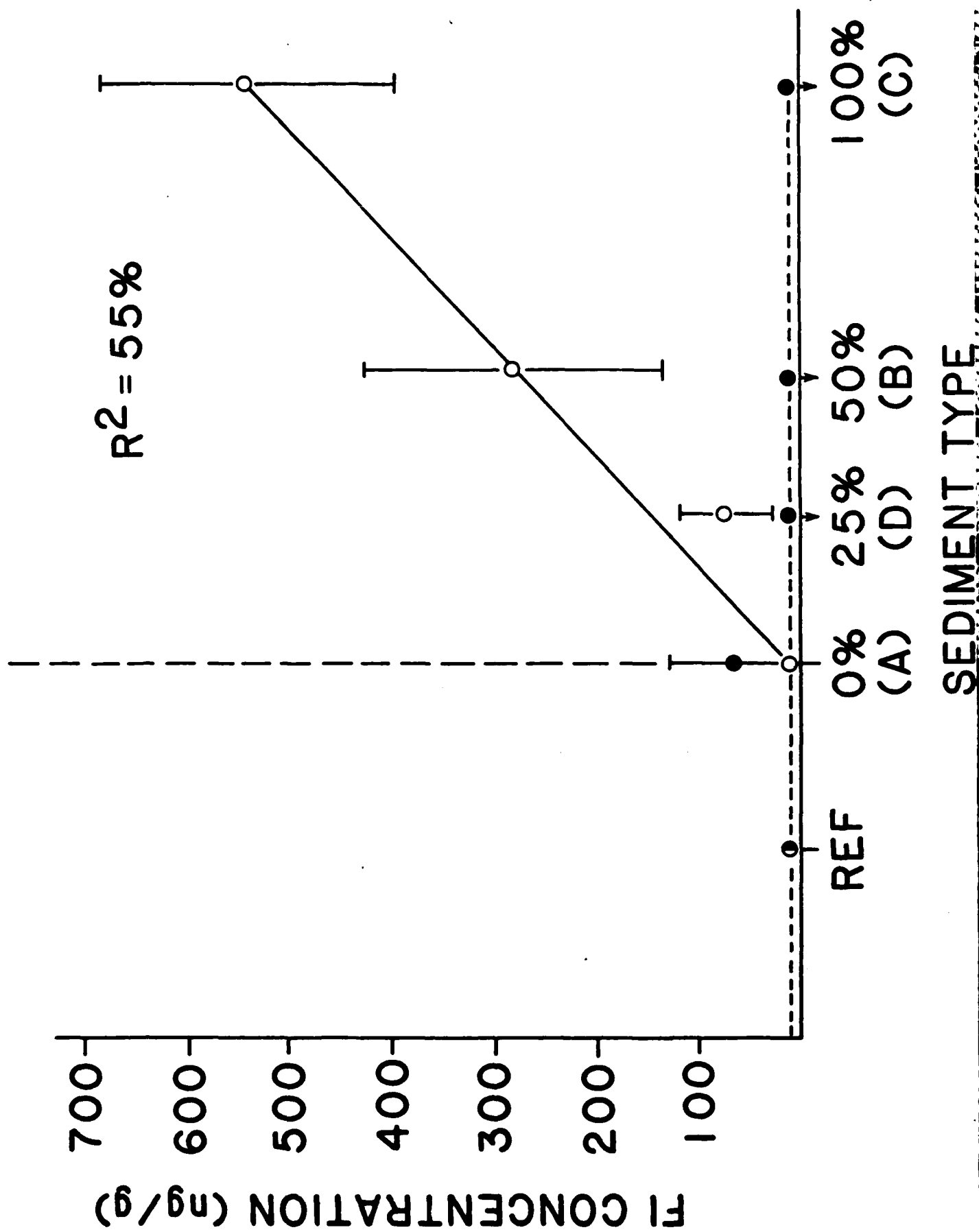
0% (A)

25% (D)

50% (B)

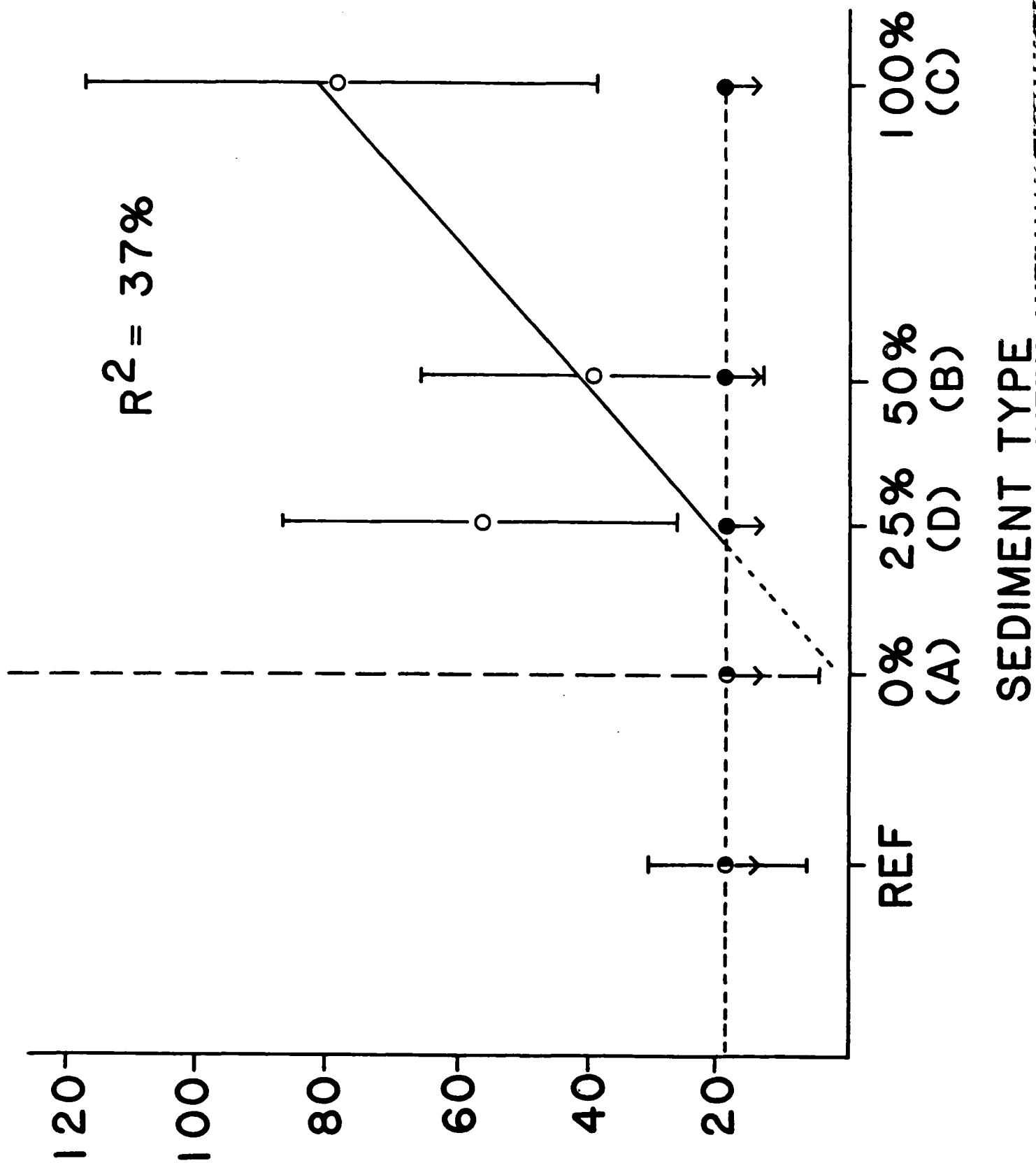
100% (C)

SEDIMENT TYPE

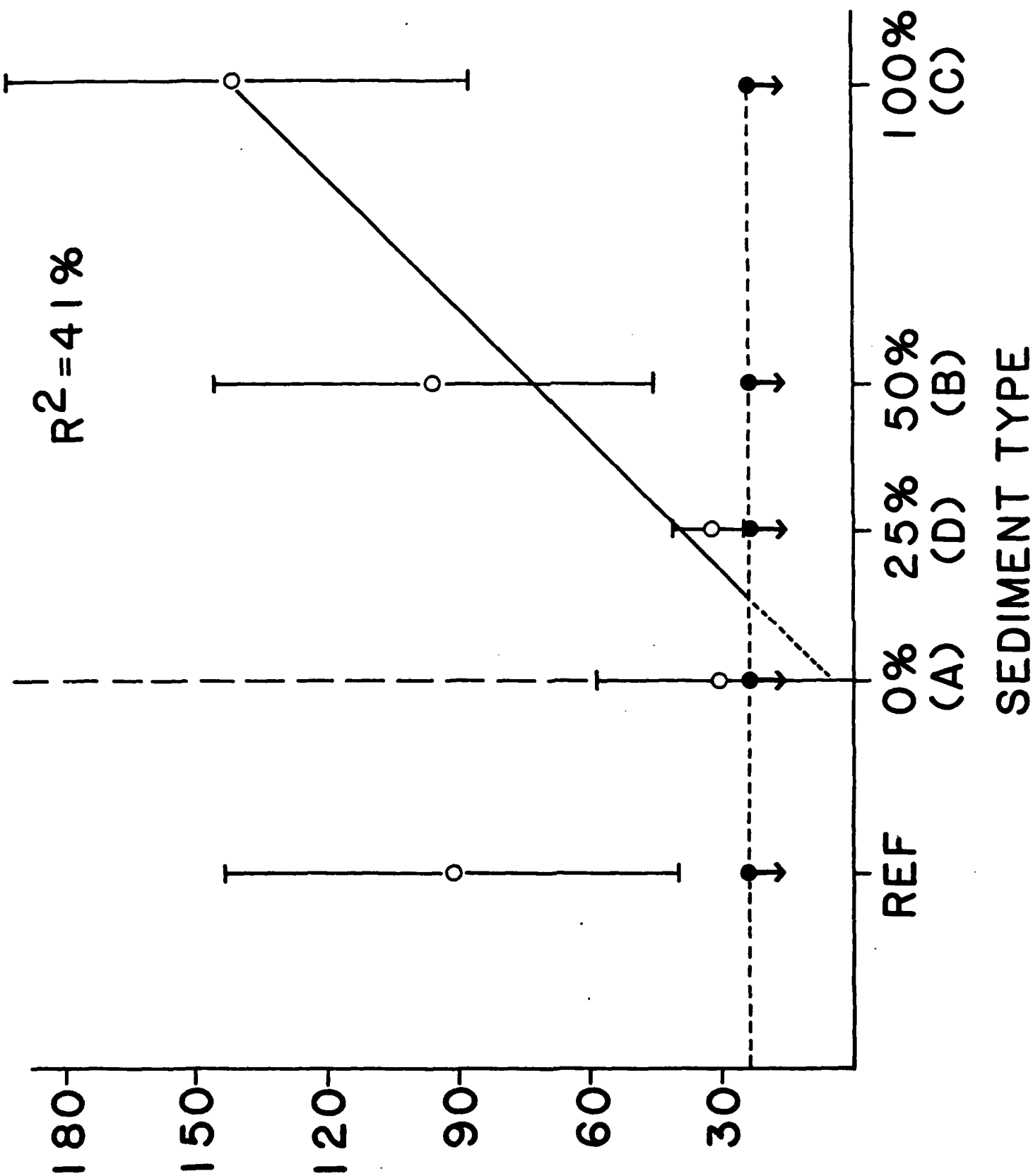


B(a)A CONCENTRATION (ng/g)

$R^2 = 37\%$



Ch CONCENTRATION (ng/g)



## DISCUSSION

### Biological Effects

The most surprising finding of the present study was that the entire sediment series displayed a low degree of acute toxicity for organisms in both the bioassays and microcosms. The lack of toxicity was particularly unexpected because high mortalities were observed in previous suspended solid and solid phase tests of sediments from the region (Alden et al., 1981; Alden and Young, 1982; Alden and Young, 1984). Recent dredging activities between 1981 and 1982 apparently removed much of the contaminants responsible for the previously observed lethality. During the 1983 tests, both the biological effects (Alden et al., 1984; Alden and Young, 1984) and the contaminant load of PNAH's (Alden and Hall, 1984) increased but not to the pre-dredging levels.

The only test organism to display elevated mortalities was the copepod Acartia tonsa. This species exhibited high mortalities when exposed to all suspended solid phase concentrations regardless of source. This trend is not too surprising, since A. tonsa has been shown to be sensitive to a high suspended solid load, even when the sediments were clean (Alden and Crouch, 1984). Dissolved materials in the higher concentrations of the liquid fraction of all sediments also produced lethal effects in this species. There were indications that the liquid phase of the sediments containing a higher percentage of ERS (50% and 100%) produced greater effects. Recent studies (Wilson, 1982) on the lethal and sublethal effects of



Kepone on A. tonsa indicated that this species is very sensitive to toxins, with significant sublethal effects occurring at levels in the low parts-per-trillion (ng/l) range. Materials leaching out of the silt/clays of even "clean" sediments may produce adverse effects, with greater impacts from more contaminated materials.

The results of the microcosm/bioassay comparison showed a distinct pattern for the bioaccumulation of toxins. The clam body burdens in the microcosm experiments were related to the concentration of the ERS. On the other hand, clams exposed to the same sediments exhibited no significant uptake patterns. The body burdens of the bioassay clams exposed to the sediments containing a high concentration of ERS were seldom significantly higher than those exposed to Eastern Shore sediments, and often not higher than those of the reference organisms.

### Heavy Metals

Heavy metals are often concentrated in sediments due to the process of sorption on fine inorganic particles and detritus as well as in association with iron and aluminum hydrous oxides. Contaminated sediments may become reintroduced to the water column through dredging activities. It is generally felt that when these contaminants are present in high concentrations in the sediment and interstitial waters, adverse impacts may be associated with the perturbations of dredged material disposal into open waters (Jones et al., 1979).

The bioassay procedure was developed by the EPA and ACOE to

assess the biological impact of dredge materials. The procedure allows for physical contact between the sediment and test species. However, static bioassays have only achieved limited success in demonstrating significant bioaccumulation of metals (Hirsch et al., 1978; Neff et al., 1978; Engler, 1978, 1980; Allen and Hardy, 1980; Peddicord and Hansen, 1983; Rubenstein et al., 1983; Alden et al., 1985, etc.).

The metal levels of sediments in portions of the Elizabeth River have been classified as being moderately high to high (U.S. EPA, 1976; Alden et al., 1981). In fact, some metal concentrations in the Elizabeth River were substantially higher than those reported in sediments from the New York Harbor, an area considered contaminated (Lee et al., 1978). The lead in New York Harbor sediments (NYHS) ranged from 8.9 to 84  $\mu\text{g/g}$ , while the level in the ERS composite was 160  $\mu\text{g/g}$ . The maximum Zn concentration in NYHS was 140  $\mu\text{g/g}$  and 472  $\mu\text{g/g}$  in ERS. The Cd and Cu maximums were higher in the NYHS than the ERS, while Cr, Ni, and Mn levels were similar. The iron was somewhat higher in the ERS. The metal levels were significantly higher in ERS than Eastern Shore sediment, with the exception of Ni and Fe, which were similar. The reference sediment from the Norfolk Disposal Site had very low levels. However, the dredging operations during 1981 lowered the sediment levels of most metals by factors of 20-50% below the levels reported for the region during the previous year (Alden et al., 1981).

No significant bioaccumulation in bioassay-exposed clams was demonstrated for any of the metals examined in the microcosm-bioassay comparison. Apparently, high sediment levels are not

always relevant to organismal uptake. Numerous reasons have been proposed for the bioaccumulation insensitivity observed in this study, as well as in others previously mentioned. In situ research has been generally unsuccessful in demonstrating organismal metal bioaccumulation over sediment levels (Cross et al., 1970; and Bryan and Hummerstone, 1971). It has been concluded that most toxins are bound to the sediment and/or are in a form that is not biologically available (Jones et al., 1979). Lee et al. (1977) criticized using bioassays for organismal toxin bioaccumulation because the procedure was too short-sighted. They felt that bioaccumulation must be examined in terms of the concentration in numerous trophic levels in the region of concern. The Lee et al. (1977) study also questioned the use of molluscs for determining bioaccumulation potential. Molluscs are reported to go into a resting phase during unfavorable conditions such periods may last for days at a time. Therefore, under highly toxic conditions, sediment toxicity and bioasscumulation potential can be greatly underestimated.

The data from the microcosm-exposed organisms on the microcosm-bioassay comparison did show significant and selective uptake for Cu, Zn, Fe and to a lesser extent Mn. These results are contrary to previous dredged sediment bioaccumulation data. Several reasons are proposed for these differences. Previous research indicates that the metals bioaccumulated in the microcosm-clams (Cu, Zn, Fe and Mn) are released to the water column during dredging or simulated operations (Lee et al., 1978; and Pequegnat et al., 1978). The water circulating devices in the microcosm

barrels kept material suspended for a longer period of time. Such circulating devices are not practical in the bioassay-design where 75% of the water is replaced at regular intervals. This quickly removes most suspended matter and any associated toxins from the bioassay testing chambers. In addition, phytoplankton and zooplankton populations included the microcosm experiments serve as a natural food source which are carried to the clams by current designed to match those measured in the field. Therefore, the more "natural" environment of the microcosms would be more conducive to normal clam behavior (e.g. feeding, burrowing, purging and respiratory activities). The clams apparently accumulate the metals (via the digestive system, gills, or integument) during these "normal" activities in the microcosms rather than "shutting down" when exposed to the contaminated sediments in static conditions of bioassays.

The levels of metals in the bioassay clams were very similar to those observed in an intensive series of bioaccumulation experiments on sediments collected from throughout the Port (Alden et al., 1985). In the Alden et al. (1985) study, a bioassay protocol was also employed and none of the 19 sites tested produced significant bioaccumulation of metals in the experimental clams above control levels. On the other hand, the microcosm clams exposed to the 100% ERS during the present study exhibited significant uptake of Cu, Zn, Fe and Mn. Results were 2-5 times the concentrations found for any of the clams examined in the intensive bioaccumulation series. This pattern tends to indicate that the bioassay protocols employed in previous studies in which (Hirsch et al., 1978; Neff et al., 1978; Engler, 1978, 1980; Allen

and Hardy, 1980; Peddicord and Hansen, 1983; Rubenstein et al., 1983; Alden et al., 1985) may have underestimated the full bioaccumulation potential of the dredged materials under more natural conditions. Fortunately, the concentrations observed in the microcosm clams were far below the lowest body burden levels shown to produce any significant biological effects (Dillon, 1984).

### **Polynuclear Aromatic Hydrocarbons**

Polynuclear aromatic hydrocarbons are a class of organic toxins from numerous sources: petroleum products, coal, creosote, and the incomplete combustion of fossil fuels (e.g. automobile exhausts, industrial smoke stacks, home heating, incinerators, etc.), among others (EPA, 1979). Surveys of the Elizabeth River have revealed that high concentrations of PNAH's (i.e. high ppm range) are found in the sediments of certain areas. The collection sites of the present study were located in a region which have been highly contaminated with PNAH's from creosote industries and shipyard activities (Alden and Hall, 1984). The high levels of these contaminants are of particular environmental concern because they are long-lived toxins and many are mutagenic and/or carcinogenic.

The levels of PNAH's in the sediments of the collection area are exceeded by only a very small percentage of values reported for samples collected world-wide (Alden and Hall, 1984). However, the 1981 dredging operations did dramatically decrease their concentrations by 1-2 orders of magnitudes (Alden and Hall, 1984).

It is quite likely that the decreased toxicity observed during the present study is associated with the reduced PNAH concentrations (Alden and Hall, 1984; Alden et al., 1984; Alden and Young, 1984). However, subsequent studies have shown that the PNAH's and the associated toxicity returned in 1983.

The potential bioaccumulation of PNAH's from dredged materials has been poorly studied. Alden et al. (1985) review the literature and discuss the dynamics of PNAH uptake in clams exposed in solid phase bioassay tests of sediments taken from throughout the Port. The bioassay clams from the present study did not show any significant uptake of PNAH's. The microcosm clams exposed to high concentrations of ERS did display significant uptake patterns for phenanthrene, fluoranthene, benzanthrane, and chrysene. This is despite the fact that the PNAH's in the sediments were reduced in 1982. These were the same PNAH's which were shown by Alden et al. (1985) to have the greatest bioaccumulation potential in most test species. The levels of these compounds were also of the same order of magnitude as the concentrations observed in clams tested during 1983, when PNAH's in the sediments had increased. In fact, only the body burdens of phenanthrene and fluoranthene were shown to be statistically significant for clams in the 1983 tests.

It may be suggested that microcosm conditions may have more effectively detected bioaccumulation of other PNAH's (e.g. benzanthrane, chrysene, pyrene, etc.) when these contaminants returned to the sediments of the region. The levels of PNAH's in the microcosm clams were higher than the body burdens of similar organisms taken from "contaminated" environments (Pancirov and

Brown, 1977; Anderson, 1979; Pancirov et al., 1980; and Murray et al., 1981). Therefore, the levels which may have been accumulated in the clams if the microcosm experiments has been conducted prior to dredging (or following a longer period of "re-invasion" of the contaminants) could have been much higher and the "impact potential" much greater. It is important to note, however, that the present study did demonstrate that the microcosm protocol was much more effective at characterizing the bioaccumulation potential, even when the toxicity of the sediments had been depleted by dredging operations. The conditions of this experiment have provided a more rigorous and realistic evaluation of the effectiveness of the technique, since most sediments in ports are not nearly as toxic as those of the middle reach of the Southern Branch prior to dredging.

## SUMMARY AND CONCLUSIONS

A side by side blind comparison was made to determine the effectiveness of bioassay and microcosm techniques. The most obvious finding of the study was that maintenance dredging had reduced the toxicity of the sediments resulting in no lethal effects by either of the techniques. The only exception was the bioassays with Acartia tonsa. It confirmed previous observations that this copepod may not be an appropriate test species for sediments containing a high silt/clay content. It is too sensitive to suspended solid effects, regardless of the relative sediment toxicity.

The most interesting conclusion from the comparison was the observed bioaccumulation pattern for toxins in the clams. Those clams exposed to microcosm sediments correctly identified the "expected" toxicity pattern of the dilution series. The bioassay clams displayed no significant uptake of the contaminants. These results reflect the fact that the more natural conditions in the microcosms stimulate activity in the bivalves that would otherwise enter a resting phase when exposed to contaminated sediments under static conditions. Since the sediments employed for both of the experiments were less contaminated than expected, it is felt that the comparison represented a more rigorous test of the effectiveness of the two techniques at detecting bioaccumulation potential in the field. The microcosm clearly detected the "correct" contamination pattern for most toxins, while the bioassays showed no uptake. This "bias" should be taken into account when solid phase bioassays are employed for assessments of the bioaccumula-



tion potential of dredged materials. A re-examination of the standard experimental design for this type of assessment is strongly suggested.

## ACKNOWLEDGEMENTS

The authors express their deepest gratitude to the numerous research assistants and field personnel who made this project successful. Particular thanks go to Roger Everton, Jeff Jewell, Phyllis Friello, and Theresa Breschell. Appreciation is extended to Sue Cooke from the Center for Instructional Development for the figures and graphs.

In addition, a special thanks goes to the Army Corps of Engineers and their field personnel who worked so hard in the water collections.

This research was supported by the Department of the Army, Norfolk District Corps of Engineers, Contract Number DACW65-81-C-0051.

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